Childhood Exposure to Passive Smoking and Bone Health in Adulthood : The Cardiovascular Risk in Young Finns Study

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Passive smoking and bone health

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Context: Passive smoke exposure has been linked with the risk of osteoporosis in adults.
Objective: We aimed to examine the independent effects of exposure to passive smoking in childhood on adult bone health.
Design/Setting: Longitudinal, the Cardiovascular Risk in Young Finns Study
Participants: Study cohort included 1422 individuals followed up for 28 years since baseline in 1980 (age 3-18 years). Exposure to passive smoking was determined in childhood. In adulthood, peripheral bone traits were assessed with quantitative computed tomography

(pQCT) at the tibia and radius, and calcaneal mineral density was estimated with quantitative ultrasound. Fracture data was gathered by questionnaires.
Results: Parental smoking in childhood was associated with lower pQCT derived bone sum index in adulthood (β±SE -0.064±0.023 per smoking parent, P=0.004) in multivariate models adjusted for age, sex, active smoking, BMI, serum 25-OH vitamin D concentration, physical activity, and parental socioeconomic position. Similarly, parental smoking was associated with lower heel ultrasound estimated bone mineral density in adulthood (β±SE -0.097±0.041 per smoking parent, P=0.02). Parental smoking was also associated with the incidence of low-energy fractures (odds ratio 1.28, 95% confidence interval 1.01-1.62). Individuals with elevated cotinine levels (3-20 ng/ml) in childhood had lower bone sum index with pQCT (β±SE -0.206±0.057, P=0.0003). Children whose parents smoked and had high cotinine levels (3-20 ng/ml) had significantly lower pQCT derived bone sum index compared to those with smoking parents but low cotinine levels (<3ng/ml) (β±SE -0.192±0.072, P=0.008).
Conclusions and relevance: Children of parents who smoke have evidence of impaired bone health in adulthood.

In this longitudinal 28-year follow-up study including 1422 individuals, parental smoking and elevated cotinine levels in childhood were associated with lower bone mineral density in adulthood. .

Introduction

Osteoporosis is a chronic systemic skeletal disease associated with elevated bone fracture risk due to a reduction in bone mass and alterations in bone quality. It is becoming an increasing public health concern along with aging populations[1]. Osteoporosis annually contributes to approximately 10 million fractures[2]. Although these fractures mostly occur in elderly people, the risk of osteoporosis may be influenced by early life exposures effecting the growing bone[3]. Therefore, it is important to identify the determinants of bone health. In prior studies, childhood growth/adiposity, physical activity and socioeconomic position have been related with bone quantity and quality[4-6].

An important environmental factor linked with osteoporosis in adults is exposure to tobacco smoke. The effects of smoking are cumulative over time, with an additional bone loss (independent of body weight and physical activity) of 4% by age 70, 6% by age 80, and 8% by age 90 years[7]. Meta-analyses have reported an increased fracture risk related to smoking in both men and women[7-9]. Importantly, exposure to secondhand smoke, i.e. passive smoking, [10-12] has also been associated with osteoporosis. However, little is known of the bone health effects of childhood exposure to passive smoking. One retrospective study in premenopausal women suggested a link between self-reported passive smoking in adolescence and reduced bone mass in adulthood[12].

In the present study, we aimed to examine passive smoking exposure in childhood (age 3-18 years) as a determinant of bone health at the skeletal maturity in mid-adulthood (age 31-46 years) among 1422 individuals. The analyses were performed in the longitudinal Cardiovascular Risk in Young Finns Study with data on peripheral bone traits of radius, tibia and calcaneus assessed with quantitative computed tomography[13] and heel ultrasonography[14], and questionnaire based information on low-energy fractures.

Materials and methods

Description of the Cardiovascular Risk in Young Finns Study has been published previously[15]. The study was approved by the institutional ethics committees, and written informed consent was obtained from all the study participants or their parents. Present analysis included 1422 participants who had a baseline evaluation during childhood in 1980 and a follow-up bone health examination 28 years later in adulthood. To control for active

smoking in childhood and adolescence, the analyses were conducted after excluding the participants who were active smokers during the baseline evaluation. Detailed methods are provided in the Supplementary Appendix[16].

Childhood exposure measures – parental smoking and serum cotinine

Parents of participants self-reported their smoking habits at baseline[17]. One parent responding on behalf of both parents was asked to indicate the smoking status separately of the mother and father in the household from two questions. The first question was whether mother/father had ever smoked daily for at least one year (responses "Yes" or "No"), and the second question whether mother/father were currently smoking (responses "does not smoke", "occasionally", "daily"). Mothers or fathers indicating they had ever smoked daily for at least one year were designated as ever smokers. Mothers or fathers that indicated currently occasionally or daily smoking were designated as current smokers.

Serum samples were collected in 1980 and stored at -20°C until they were analyzed in 2014. During storage, samples were not thawed or refrozen. Serum cotinine measures were performed using standardized methods[18]. Cotinine values between 3-20 ng/ml in non-smokers were considered as indicative of positive nicotine exposure indicative of passive smoking (the concentration that could be detected reproducibly in the assay).

A three-level variable to indicate parental smoking hygiene was constructed as follows: 1 = no parental smoking and non-detectable cotinine level; 2 = parental smoking and non-detectable cotinine level (hygienic parental smoking); and 3 = parental smoking and detectable serum cotinine (non-hygienic parental smoking).

Adult outcome variables - bone traits

Peripheral quantitative computed tomography (pQCT) (Stratec XCT 2000R, Germany) was used to scan two sites (distal and diaphysis) of radius and tibia[19]. This method provides data on volumetric bone mineral density (vBMD). The following bone traits were measured: total bone mineral content (mass), total and cortical bone areas, and trabecular and cortical bone densities. In addition, three bone strength indices were estimated. From these data, four composite indices of bone mass, density, area and strength were calculated. Additionally, the four indices were combined into one age- and sex-standardized bone sum index. In attrition analyses comparing baseline characteristics between those attending (N=1800) and those non-attending (N=1796) the bone study, it was observed that non-attendants were younger (10.0 vs 10.9 years, P<0.001) and more often males (55 vs 43%, P<0.0001). However, there were no differences in baseline BMI (17.8 vs. 17.9 kg/m^2, P=0.20) or parental school years (10.7 vs 10.7 years, P=0.97). As another variable, we measured heel bone traits with a quantitative ultrasound device (Sahara Clinical Bone Sonometer, Hologic, Waltham, MA, USA) among 1210 participants. It measures the speed of sound and ultrasound attenuation at the mid-calcaneus as the sound waves traverse through bone tissue. The speed of sound (m/s) is linearly dependent on bone mineral density[20]. Additionally, an estimate of heel bone mineral density Z-score was calculated as the difference from the age- and sex-specific averages. Persons performing bone measurements were blinded to the parental smoking status. Detailed description of measurement and calculation of bone traits is given in the Supplementary appendix[16].

Fractures

During the bone study visit, participants were inquired their fracture history. Questionnaire information on fracture site, fracture age, and how the fracture occurred were collected. Fractures were classified as low-energy fractures if they were sustained in standing positions, and in the absence of excess strain due to falling from heights greater than standing level or due to the high speed of a vehicle used (cycling, skiing, skating), or if no other person or external factor was involved.

Covariates

To control for the effect of body size, we utilized serial data on height and weight collected in clinical examinations in 1980, 1983 and 1986 to calculate the estimated area under the body mass index curve between ages 6 and 24 years[21]. Data collected in study years 1980, 1983 and 1986 were used to estimate physical activity in childhood, adolescence and young adulthood. At ages 3 and 6 years, a preschool children physical activity index was calculated from the parents' ratings of the amount and vigorousness of their child's play time and the child's general level of activity as compared with other children[22]. At ages 9-24 years data on frequency and intensity of physical activity during leisure time were acquired with a self-administered questionnaire and a sum index of physical activity was calculated[23]. The values for physical activity indices were standardized and the average value was used as a measure of physical activity exposure during the time of peak bone mass accrual. As a marker of nutritional vitamin D status, the baseline (year 1980) circulating 25-OH vitamin D concentration was measured using radioimmunoassay (DiaSorin, Stillwater, MN). In adulthood, information on smoking was collected with questionnaires in 2001 and 2007. Data on parental education (years) was used as an indicator of the childhood socioeconomic status. Information on birth weight was collected using questionnaires and confirmed from participants' records from well-baby clinics.

Statistical analysis

Statistical analysis was performed using SAS 9.3. Statistical significance was inferred by a P value <0.05. Parental smoking, serum cotinine, and parental smoking hygiene were used as exposure variables in multivariable linear regression models to examine effects of childhood passive smoking on pQCT and ultrasound derived bone indices. Analyses using dichotomized fracture data as an outcome were performed with logistic regression analyses. Stepwise multivariate regression with backward elimination was performed to take into account the effects of possible intermediate or confounding factors. Variables in initial stepwise multivariate models included age, sex, body mass index, physical activity, parental school years, and serum 25-OH vitamin D in childhood. Age and sex were forced into the final models. In addition, the effects of birth weight and smoking in adulthood were controlled for in additional models.

We examined potential sex and age interactions by including interaction terms in logistic regression models. In addition, we investigated differences between the effects of a smoking mother and a smoking father. Replications of the analyses were done including only life-long non-smokers. Sensitivity analyses were performed using different cut-offs for serum cotinine (2.5, 3.0, 3.5 or 4.0 ng/L) to indicate passive smoking.

Results

Baseline characteristics (in 1980) stratified by parental smoking exposure are shown in Supplementary Table 1[16]. Bone measures, the calculation of bone indices and their mean values are shown in Supplementary Table 2[16].

Parental smoking and bone health

The effect of regular parental smoking during childhood on the pQCT derived bone indices in adulthood is shown in Table 1. In multivariable models, exposure to parental smoking was statistically significantly and inversely associated with the bone sum index, bone mass, bone density and bone strain, but not with bone area. The effect estimates of parental smoking remained essentially similar when the analyses were adjusted for birth weight (N=1214) or active smoking in adulthood (N=1345), or when active smokers in adulthood were excluded (N=1135). No evidence of statistically significant sex interactions was observed. Similarly,

no significant age interactions were detected. The effect estimates were nearly identical for paternal smoking and maternal smoking.

Results for ultrasound measured bone indices are in Table 2. Parental smoking was inversely related with broadband attenuation, speed of sound and estimated bone mineral density Z-score. The findings remained essentially similar when additionally adjusted for birth weight (N=1067) or active smoking in adulthood (N=1171), or when active smokers in adulthood were excluded (N=961). There were no age-interactions, but statistically significant sex interactions were observed for all indices. In sex-stratified analyses, parental smoking was related with ultrasound derived bone indices among females (P always < 0.005), but not in males (P always > 0.6). The effect estimates were comparable for paternal and maternal smoking.

Questionnaire based low-energy fracture rates among individuals with 0, 1 and 2 smoking parents were 9.2, 12.0 and 13.7 %, respectively. Odds ratio per smoking parent was 1.28 (95% CI 1.01-1.62, P=0.04) in a logistic regression model adjusted for age, sex and childhood factors. The results remained essentially similar when the analyses were additionally adjusted for birth weight or active smoking in adulthood, but the association was attenuated when active smokers in adulthood were excluded (P=0.24).

Cotinine exposure in childhood and bone health

Passive smoking in childhood, defined as a serum cotinine concentration between 3-20 ng/ml, was inversely associated with the pQCT derived bone sum index and the bone mass, density and strength indices in adulthood (Table 3). These associations remained similar after additional adjustment for birth weight (N=1047) or smoking in adulthood (N=1195), or when active smokers in adulthood were excluded (N=995). There were no significant age or sex interactions.

Concerning ultrasound measures, cotinine exposure was inversely associated with speed of sound (Table 4). Effect estimates were not altered after additional adjustments for birth weight (N=887) or active smoking in adulthood (N=1006), or when active smokers in adulthood were excluded (N=835). No significant age or sex interactions were observed.

Those individuals with low cotinine levels had a low-energy fracture rate of 11.3 % and among those with elevated cotinine it was 14.3% (P=0.15 in a logistic regression model adjusted for age, sex and childhood factors).

Parental smoking hygiene in childhood and bone health in adulthood

Figure 1 shows the association between parental smoking hygiene and bone indices. Children whose parents smoked non-hygienically (cotinine levels in children 3-20 ng/ml) had lower pQCT derived bone sum index compared with those whose parents smoked hygienically (cotinine levels in children <3ng/ml) or those whose parents did not smoke (Figure 1). Concerning ultrasound derived estimated bone mineral density Z-score, there were no differences between the groups (Figure 2).

Sensitivity analyses and replication

In analyses restricted to life-long non-smokers results were essentially similar to those shown in Tables 1-4. There were no significant differences in the effects of passive smoking in childhood on bone traits according to different cotinine concentration cut-offs (Supplementary Table 3[16]). Results remained similar when no upper limit to cotinine concentrations was applied.

Discussion

We observed that exposure to passive smoking in childhood, determined by parental smoking and serum cotinine concentrations, was a significant determinant of reduced bone mass, density and strength indices measured 28 years later in adulthood with two different methods.

The effect of passive smoking in childhood was not attenuated after adjustments for age and sex and the possible intermediate or confounding factors, including BMI, active smoking, serum 25-OH vitamin D concentration, physical activity, parental school years and birth weight.

In adulthood, active smoking is a risk factor for osteoporosis and bone fractures[7,8,24-28]. Less is known of the effects of passive smoking on bone health. In adults, passive smoking has been inversely associated with phalangeal bone mineral density in a cohort of 15,038 adults aged 19-95 years[10]. Similar results were found in 2067 postmenopausal women, where passive smoking confirmed by urinary cotinine analysis was directly associated with osteoporosis[11]. Even less is known of passive smoking in childhood. In a retrospective study on 154 premenopausal women, self-reported exposure to passive smoking from age 10 onwards was negatively associated with total hip and femoral neck bone mineral density when aged 40-45 years[12]. The results of the present prospective study indicate that parental smoking exposure in childhood affects subsequent bone quality traits measured in mid-adulthood.

Concerning other childhood risk factors, the available prospective longitudinal studies demonstrating links between childhood exposures and adult bone health outcomes have mainly evaluated the effects of early growth, physical activity and socioeconomic position. It has been shown that poor fetal and infant growth and low levels of physical activity in childhood are associated with reduced peak bone mass later in life[29]. Direct relations have been observed between childhood overweight and adult bone density, supporting the hypothesis that excess weight during active growth imposes increased loading on the weight-bearing skeleton and leads to more robust bones in adulthood[19]. Among white males, socioeconomic disadvantage in childhood has been associated with lower adult femoral neck strength[6]. In the present analyses, the effects of childhood exposure to secondhand smoke on bone health were independent of these factors, as well as other possible confounders, such as vitamin D concentrations and family socioeconomic status.

Most plausible mechanisms in smoking-induced bone loss may be increased bone resorption and a less efficient calcium absorption[30] and effects on circulating levels of sex hormones and 25-OH vitamin D[25]. Experimental nicotine exposure inhibited matrix synthesis and hypertrophic differentiation in human growth plate chondrocytes[31]. There is a large body of evidence from experimental studies that tobacco smoke has adverse effects on osteoneogenesis and osseointegration in bone cell culture and animal models via several mechanisms[32]. In animal models of bone biomechanical properties, tobacco smoke exposure decreased the structural strength, material properties, bone mass, and trabecular quality in the growing female mouse[33], and decreased bone mineral density through increased bone turnover in the female rat[34]. Nicotine has been suggested to have a direct toxic effect on osteoblasts[35]. However, experimental nicotine administration in rats caused no differences in bone mineral content or other bone traits between the low and high nicotine doses[36,37]. Thus, substances in smoke other than nicotine may also be responsible for the decreased bone density. In the present study, independent associations were seen with different indices of bone mineral density, bone mass and strength after a 28-year follow-up in both men and women, suggesting that tobacco smoke exposure may compromise the growing bone through multiple mechanisms.

From a clinical point of view, we observed in multivariable models that parental smoking in childhood was associated with up to 0.19 SD worse (estimated heel BMD, for both parents smoking) bone measures, and elevated cotinine levels (3-20 ng/L) were related with over 0.2 SD lower bone sum index. In addition, parental smoking was related with low-energy fractures. In prospective observational data, 1-SD decrease in bone mineral density with DXA has been related to approximately 1.4 times elevated total osteoporotic fracture risk at

the age of 65 years[38]. However, for hip fractures the respective risk ratio for 1 SD change in bone mineral density is 2.9 at the age of 65 years and the relative risk significantly increases with decreasing age[38]. For these reasons, it would be essential to have increased public health awareness to the harms associated with secondhand tobacco smoke, especially in childhood. There would be several different ways to limit children's exposure to environmental tobacco smoke, including restrictions to smoking in public places, in vehicles, and at home. Smoking restrictions in public and work places have been shown to decrease hospitalizations for cardiovascular and respiratory disease among adults[39]. However, there are observational data suggesting that public smoking regulations may have increased passive smoke exposure in private places, such as at home[40]. Therefore it would be important to communicate to parents that their smoking has effects on their children's health, both in short and long term.

Our study has limitations. There was only a single measurement of parental smoking and of serum cotinine concentration in childhood at the age of 3 to 18 years. However, we did not detect age interactions, indicating that a single measurement in childhood may be representative of exposure from childhood through youth. We were unable to determine an age when exposure to parental smoking may have been most detrimental to bone health. A limitation may also be that no data were available on smoking during pregnancy which may affect birth weight, however all models were adjusted for birth weight. The present pQCT and ultrasound results performed in peripheral bones, including calculations of bone sum index, provide epidemiological data and they do not have instant clinical utility. The estimated BMDs and Z-scores are not comparable with DXA measurements and they cannot be used for diagnostic classification. Another potential limitation is the non-participation in the bone measurement study. However, even though non-participants were younger and more often males, their baseline characteristics (BMI, parental education) were similar. Thus the present study cohort seems to be representative of the original study population. The strengths of our study are the large, well characterized population with a long clinical follow-up and the bone measurement methods of assessing peripheral bone mass, density, area and strength from three different bones, radius, tibia and calcaneus. A further strength is that exposure to secondhand smoke in childhood could be confirmed by serum cotinine which is a biomarker of nicotine exposure. Furthermore, we performed the analyses excluding those who had reported own smoking at the baseline.

Our results suggest that bone traits are persistently affected by exposure to passive smoking in childhood, independent of potential confounding factors. Programs aimed at avoiding exposure to tobacco smoke early in life could improve later bone health of children in risk to passive smoke exposure.

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Conflict of interest
There is no conflict of interest.
Disclosure statement
The authors have nothing to disclose.

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Figure 1. Effect of parental smoking hygiene at the offspring's age of 3-18 years on bone sum index measured with peripheral quantitative computed tomography in adulthood (age 31-46 years). Results are expressed as mean±SEM, p-values are from regression analyses adjusted for age, sex, childhood body mass index, physical activity, parental school year and 25-OH vitamin D-concentration. Serum cotinine concentration between 3 and 20 ng/ml was considered elevated.

Figure 2. Effect of parental smoking hygiene at the offspring's age of 3-18 years on calcaneal bone mineral density Z-score estimated with ultrasound in adulthood (age 31-46 years). Results are expressed as mean±SEM, p-values are from regression analyses adjusted for age, sex, childhood body mass index, physical activity, parental school year and 25-OH vitamin D-concentration. Serum cotinine concentration between 3 and 20 ng/ml was considered elevated.

Table 1. Multivariable regression results of the independent effects of parental smoking in childhood (age of 3-18 years) on bone quality indices measured by peripheral quantitative computed tomography in adulthood (age 31-46 years) from 1422 participants (aged 31-46 years) of the Cardiovascular Risk in Young Finns Study.

	Bone sum index (z-score)		**Bone mass (mg)**		**Bone density (mg/cm^3)**		**Bone area (mm^2)**		**Bone strain (z-score)**	
	β±SE	P	β±SE	P	β±SE	P	β±SE	P	β±SE	P
Age (years)	0.016±0.003	<0.0001	5.8±1.0	<0.0001	-0.13±0.09	0.17	6.7±1.1	<0.0001	0.015±0.003	<0.0001
Male sex	1.509±0.033	<0.0001	511.2±9.4	<0.0001	4.76±0.91	<0.0001	408.5±10.4	<0.0001	1.614±0.029	<0.0001
Childhood body mass index	0.237±0.017	<0.0001	85.8±5.0	<0.0001			72.1±5.5	<0.0001	0.244±0.016	<0.0001
Childhood physical activity	0.129±0.019	<0.0001	47.4±5.4	<0.0001			35.1±6.0	<0.0001	0.110±0.017	<0.0001
Parental smoking *	-0.064±0.023	0.004	-17.1±6.4	0.008	-1.27±0.63	0.04	-11.1±7.4	0.12	-0.042±0.020	0.04

Results are from stepwise multivariable models. β = Parameter estimate for change in the outcome variable for 1-standard deviation / 1-category change in the exposure. SE = standard error. Initial models included data on age, sex, childhood body mass index, physical activity, parental school years, 25-OH vitamin D-concentration and parental smoking. Age and sex were forced into final models. * Effect per one smoking parent.

Table 2. Multivariable regression results of the independent effects of parental smoking in childhood (age of 3-18 years) on ultrasound derived bone quality indices in adulthood (age 31-46 years) from 1210 participants (aged 31-46 years) of the Cardiovascular Risk in Young Finns Study.

	Broadband attenuation (dB/MHz)		**Speed of sound (m/s)**		**Estimated bone mineral density (Z-score)**	
	β±SE	**P**	**β±SE**	**P**	**β±SE**	**P**
Age (years)	0.3±0.1	0.01	0.1±0.2	0.85	0.011±0.006	0.09
Male sex	2.3±1.0	0.02	-0.7±1.7	0.70	-0.075±0.060	0.21
Childhood body mass index	2.5±0.5	<0.0001	2.6±0.9	0.005	0.123±0.032	0.0001
Childhood physical activity	1.8±0.6	0.001	3.7±1.0	0.002	0.116±0.034	0.0009
Parental smoking *	-1.6±0.6	0.02	-2.7±1.1	0.02	-0.097±0.041	0.02

Results are from stepwise multivariable models. β = Parameter estimate for change in the outcome variable for 1-standard deviation / 1-category change in the exposure. SE = standard error. Initial models included data on age, sex, childhood body mass index, physical activity, parental school years, 25-OH vitamin D-concentration and parental smoking. Age and sex were forced into final models. * Effect per one smoking parent.

Table 3. Multivariable regression results of the independent effects childhood exposure to cotinine (age of 3-18 years) on bone quality indices measured by peripheral quantitative computed tomography in adulthood (age 31-46 years) in 1201 participants (aged 31-46 years) of the Cardiovascular Risk in Young Finns Study.

	Bone sum index (z-score)		Bone mass (mg)		Bone density (mg/cm^3)		Bone area (mm^2)		Bone strain (z-score)	
	β±SE	P	β±SE	P	β±SE	P	β±SE	P	β±SE	P
Age (years)	0.014±0.004	0.0003	5.4±1.1	<0.0001	-0.2±0.1	0.09	6.6±1.2	0.002	0.014±0.004	0.0002
Male sex	1.502±0.036	<0.0001	509.7±10.1	<0.0001	4.6±1.0	<0.0001	405.9±11.1	<0.0001	1.614±0.031	<0.0001
Childhood body mass index	0.215±0.019	<0.0001	79.4±5.4	<0.0001			73.2±5.9	<0.0001	0.226±0.017	<0.0001
Childhood physical activity	0.146±0.022	<0.0001	55.2±6.2	<0.0001			34.0±6.8	<0.0001	0.132±0.019	<0.0001
Elevated cotinine in childhood *	-0.206±0.057	0.0003	-47.6±16.0	0.003	-6.1±1.6	0.0001	-4.1±17.6	0.81	-0.149±0.050	0.003

Results are from stepwise multivariable models. β = Parameter estimate for change in the outcome variable for 1-standard deviation / 1-category change in the exposure. SE = standard error. Initial models included data on age, sex, childhood body mass index, physical activity, parental school years, 25-OH vitamin D-concentration and parental smoking. Age and sex were forced into final models. *Serum cotinine concentration between 3 and 20 ng/ml

Table 4. Multivariable regression results of the independent effects childhood exposure to cotinine (age of 3-18 years) on ultrasound derived bone quality indices in adulthood (age 31-46 years) in 1011 participants (aged 31-46 years) of the Cardiovascular Risk in Young Finns Study.

	Broadband attenuation (dB/MHz)		Speed of sound (m/s)		Estimated bone mineral density (Z-score)	
	β±SE	**P**	**β±SE**	**P**	**β±SE**	**P**
Age (years)	0.4±0.1	0.001	0.2±0.2	0.34	0.016±0.007	0.02
Male sex	2.0±1.0	0.05	-1.2±1.9	0.53	-0.079±0.065	0.22
Childhood body mass index	2.3±0.5	<0.0001	2.4±1.0	0.02	0.115±0.035	0.001
Childhood physical activity	3.0±0.6	<0.0001	5.8±1.2	<0.0001	0.187±0.041	<0.0001
Elevated cotinine in childhood *	-2.2±1.6	0.16	-7.0±2.9	0.01	-0.184±0.099	0.06

Results are from stepwise multivariable models. β = Parameter estimate for change in the outcome variable for 1-standard deviation / 1-category change in the exposure. SE = standard error. Initial models included data on age, sex, childhood body mass index, physical activity, parental school years, 25-OH vitamin D-concentration and parental smoking. Age and sex were forced into final models. *Serum cotinine concentration between 3 and 20 ng/ml